H301-NIKON-NZ100/200/500-N

Compatible with the following XY stages

- Mad City Labs NZ100/200/500-N on Nikon mechanical or motorized stages, TI-S-E and TI-S-ER.

Compatible with the following Okolab Controllers

- H301-T-BL-PLUS
- UNO-COMBINED-CONTROLLER
- H401-T-DUAL

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1. Components and dimensions

H301-NIKON-NZ100/200/500-N includes the following components:

- **Chamber main body** with embedded temperature sensor
- **Sliding heated glass lid** with Indium Tin Oxide (ITO) conductive coating and embedded temperature sensor
- **Chamber riser**. It is a removable frame increasing the height of the chamber from 27 to 33 mm, often required when using multi-well (MW) plates

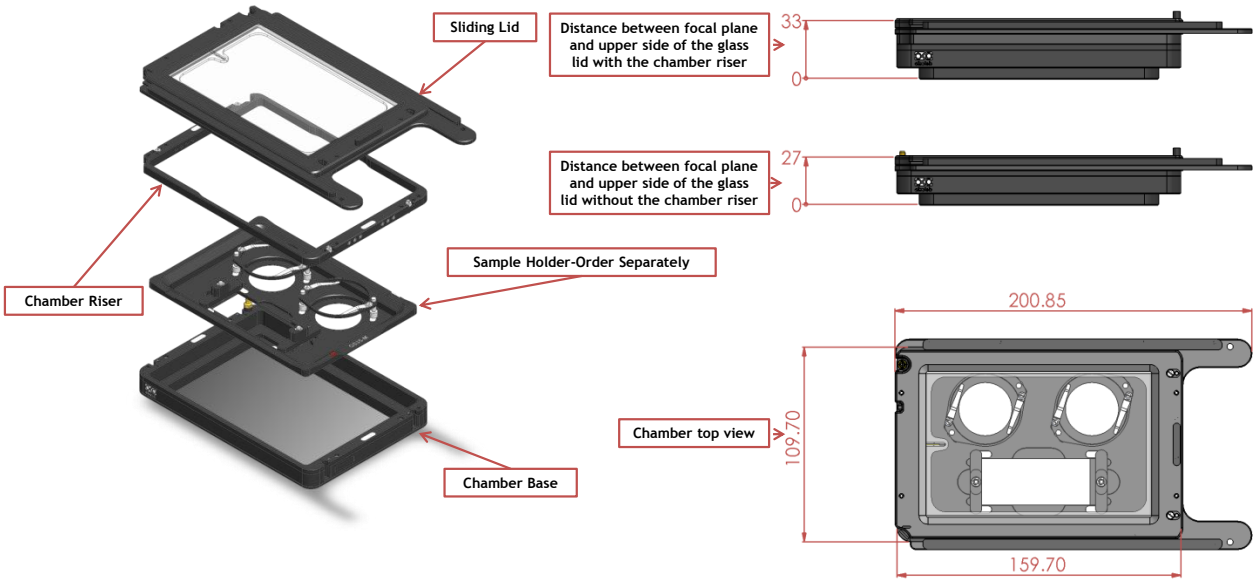

Figure 1. H301-NIKON-NZ100/200/500-N. Components and Dimensions.

2. Sample Holders

2.1 Available Sample Holders

The following sample holders are available.

NOTE: Please contact info@oko-lab.com if you cannot find the sample holder you are looking for. We are constantly adding new inserts to the list.

1x35-M	#1 35mm Petri-dish
1xGS-M	#1 1x3in. chamber slide
1xLABTEK-M	#1 Lab-Tek 1in.x2in. chambered cover glass
1xLABTEK-II-M	#1 Lab-Tek II 1in.x2in. chambered cover glass
1x60-M	#1 60mm Petri-dish
1xT25-M	#1 Nunc and Greiner T25 flask
2x35-M	#2 35mm Petri-dish
2xGS-M	#2 1x3in. chamber slides
2xLABTEK-M	#2 Lab-Tek 1in.x2in. chambered cover glass

2xLABTEK-II-M	#2 Lab-Tek II 1in.x2in. chambered cover glass
2x60-M	#2 60mm Petri-dish
4x35-M	#4 35mm Petri-dish
GS35-M	#1 1x3in. chamber slide and #2 35mm Petri-dish
LABTEK-35-M	#1 Lab-Tek 1in.x2in. chambered cover glass and #2 35mm Petri-dish
LABTEK-II-35-M	#1 Lab-Tek II 1in.x2in. chambered cover glass and #2 35mm Petri-dish
LABTEK-60-M	#1 Lab-Tek 1in.x2in. chambered cover glass and #1 60mm Petri-dish
LABTEK-II-60-M	#1 Lab-Tek II 1in.x2in. chambered cover glass and #1 60mm Petri-dish
GS60-M	#1 1x3in. chamber slide and #1 60mm Petri-dish
6035-M	#1 60mm Petri-dish and #1 35mm Petri-dish
6MW	6-well plates, one MW LOCK required
12MW	12-well plates, one MW LOCK required
24MW	24-well plates, one MW LOCK required
24MW-NUNC	24-well NUNC/GREINER plates, one MW LOCK required
48MW	48-well plates, one MW LOCK required
96MW	96-well plates, one MW LOCK required. It includes the insert for oil immersion imaging.
MW-LOCK-22.5	Magnetic lock to hold the standard MW plates in the chamber
MW-LOCK-16.5	Magnetic lock to hold the low-profile MW plates in the chamber

GS35-M
For #1 1"x3"chamber slide and #2 35mm petri dishes
4x35-M
For #4 35 mm petri dishes
2x60-M
For #2 60 mm petri dishes
GS60-M
For #1 60mm petri dish and #1 1"x3"chamber slide
1x60-M
For #1 60 mm petri dish
1x35-M
For #1 35 mm petri dish
6035-M
For #2 35 mm petri dishes and #1 60mm petri dish
1xGS-M
For #1 1"x3"chamber slide
2xGS-M
For #2 1"x3"chamber slides
MW OIL
For oil immersion
With all types of well-plates
6MW
For #1 6-well plates holder
12MW
For #1 12-well plates holder
24MW
For #1 24-well plates holder
24-NUNC-MW
For #1 24NUNC/GREINER-well plates holder
48MW
For #1 48-well plates holder
96MW
For #1 96-well plates holder
LABTEK-35-M
For #1 Lab-Tek 1"x2"chambered cover glass and #2 35mm petri dishes
LABTEK-60-M
For #1 Lab-Tek 1"x2"chambered cover glass and #1 60mm petri dish
1xLABTEK-M
For #1 Lab-Tek 1"x2"chambered cover glass
2xLABTEK-M
For #2 Lab-Tek 1"x2"chambered cover glass
1xGS-2xLABTEK-M
For #1 1"x3"chamber slide and #2 Lab-Tek 1"x2"chambered cover glass
LABTEK-II-35-M
For #1 Lab-Tek II 1"x2"chambered cover glass and #2 35mm petri dishes
LABTEK-II-60-M
For #1 Lab-Tek II 1"x2"chambered cover glass and #1 60mm petri dish
1xLABTEK-II-M
For #1 Lab-Tek II 1"x2"chambered cover glass
2xLABTEK-II-M
For #2 Lab-Tek 1"x2"chambered cover glass
1xGS-2xLABTEKII-M
For #1 1"x3"chamber slide and #2 Lab-Tek II 1"x2"chambered cover glass
2x35-M
For #2 35 mm petri dishes
1xT25-M
For #1 Nunc and Greiner T25 flask holder
3xGS-M
For #3 1"x3"chamber slide

Figure 2.Available sample holders.

3. Available Lids

The following heated glass Lids are available for H301-NIKON-NZ100/200/500-N:

- **H301-SLIDING-LID**: For easy sample loading and pipetting – **Included in code** H301-NIKON-NZ100/200/500-N. Compatible with Mad City Labs NZ100/200 ONLY.
- **H301-KOEHLER-LID***: Reduces chamber height to 22 mm and allows imaging under Koehler illumination
- **H301-INJECTION-LID***: Heated glass lid with two small openings (sealed with flexible plastic) allowing injection or permanent access to the sample. Compatible only with sample holder 1x35
- **H301-LASER-INTERLOCK-LID***: Heated glass lid with safety switch. Connects to laser controller and automatically turns laser off when lid is lifted. Compatible only with sample holder 1x35

* OPTIONAL – not included with H301-NIKON-NZ100/200/500-N

3.1 Sliding lid

The Sliding Lid is a heated glass lid allowing for easy sample loading and pipetting. The Sliding Lid is screwed onto the chamber.

NOTE:H301-SLIDING-LID is compatible with Mad City Labs NZ100/200 ONLY.

Figure 3 shows chamber dimensions with the Sliding Lid (with and without chamber riser). Figure 4 illustrates how to remove Sliding Lid, when a different Lid is necessary.

NOTE: Sliding Lid MUST BE REMOVED when using any other Lid. The Sliding Lid is fixed onto the chamber with 4 screws. Screws location is indicated by letter A in Figure 4 (image 2 and 3). Keep Sliding Lid fully closed to access screws labeled A in image 2. Open Sliding Lid to access screws labeled A in image 3.

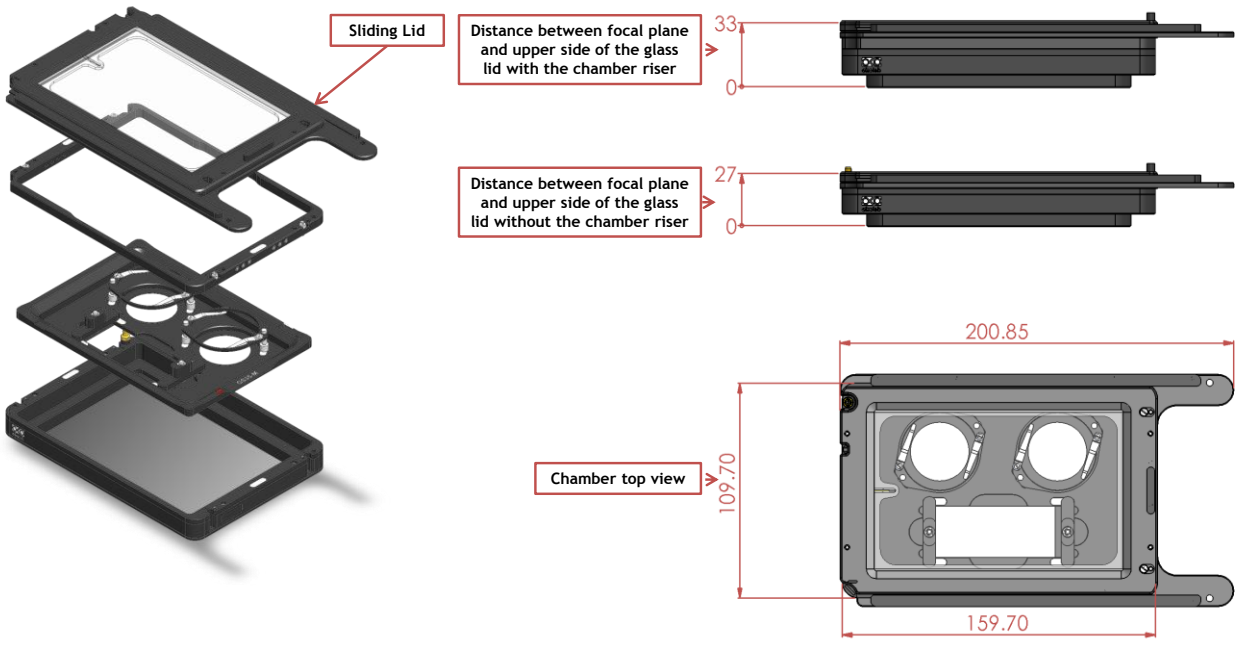

Figure 3. H301-NIKON-NZ100/200/500-N (Sliding Lid comes as standard)

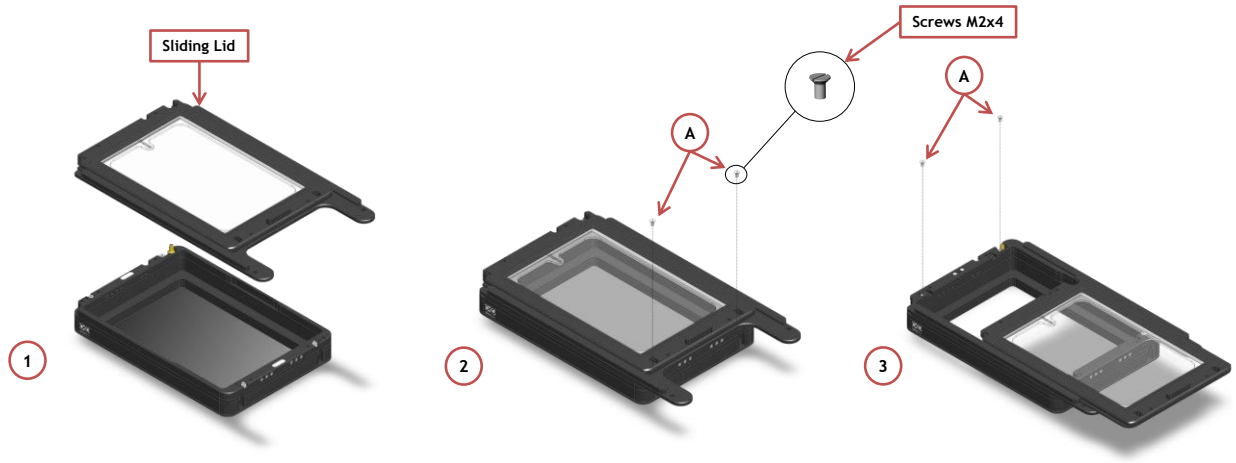

Figure 4. Assembly of the sliding lid

3.2 Koehler Lid

The Koehler Lid is a heated glass lid reducing the chamber's height to 22 mm and allowing imaging under Koehler illumination. The Koehler Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 5 shows chamber dimensions with Koehler lid (with and without chamber riser).

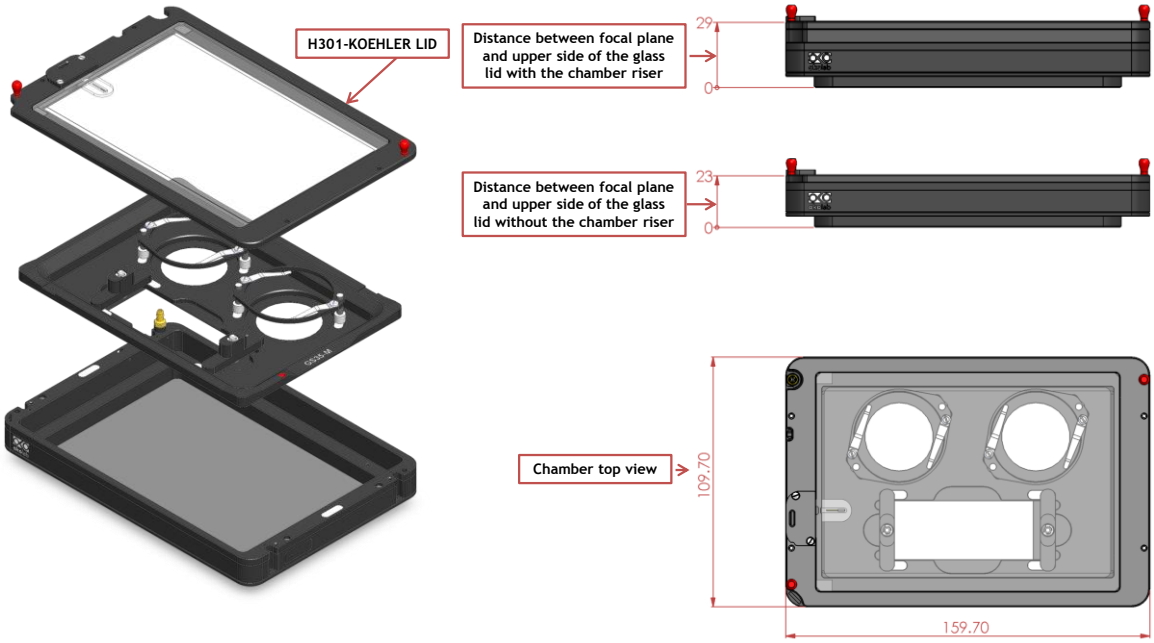
H301-KOEHLER LID
Distance between focal plane and upper side of the glass lid with the chamber riser
29
0
Distance between focal plane and upper side of the glass lid without the chamber riser
23
0
Chamber top view
109.70
159.70

Figure 5. H301-NIKON-NZ100/200/500-N + H301-KOEHLER-LID

3.3 Injection Lid

The Injection Lid is a heated glass lid with two small openings sealed with flexible plastic allowing injection or permanent access to the sample. NOTE: Compatible with sample holder 1x35 ONLY. The Injection Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 6 shows chamber dimensions with the Injection Lid (with and without chamber riser).

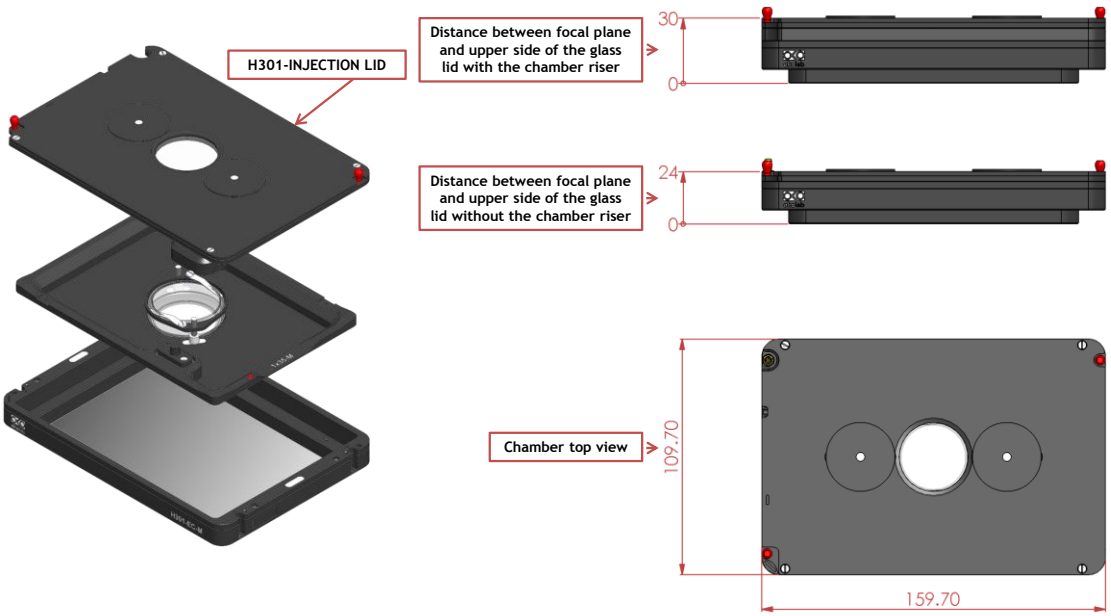
H301-INJECTION LID
Distance between focal plane and upper side of the glass lid with the chamber riser
30
0
Distance between focal plane and upper side of the glass lid without the chamber riser
24
0
Chamber top view
109.70
159.70

Figure 6. H301-NIKON-NZ100/200/500-N + H301-INJECTION-LID

3.4 Laser Interlock Lid

The Laser Interlock Lid is a heated glass lid with a safety switch connecting to the laser controller. It automatically turns the laser off when the lid is lifted. NOTE: Compatible with sample holder 1x35 ONLY. The Laser Interlock Lid is placed onto the chamber (NO SCREWS or TOOLS are necessary).

Figure 7 shows chamber dimensions with the Laser Interlock Lid (with and without chamber riser).

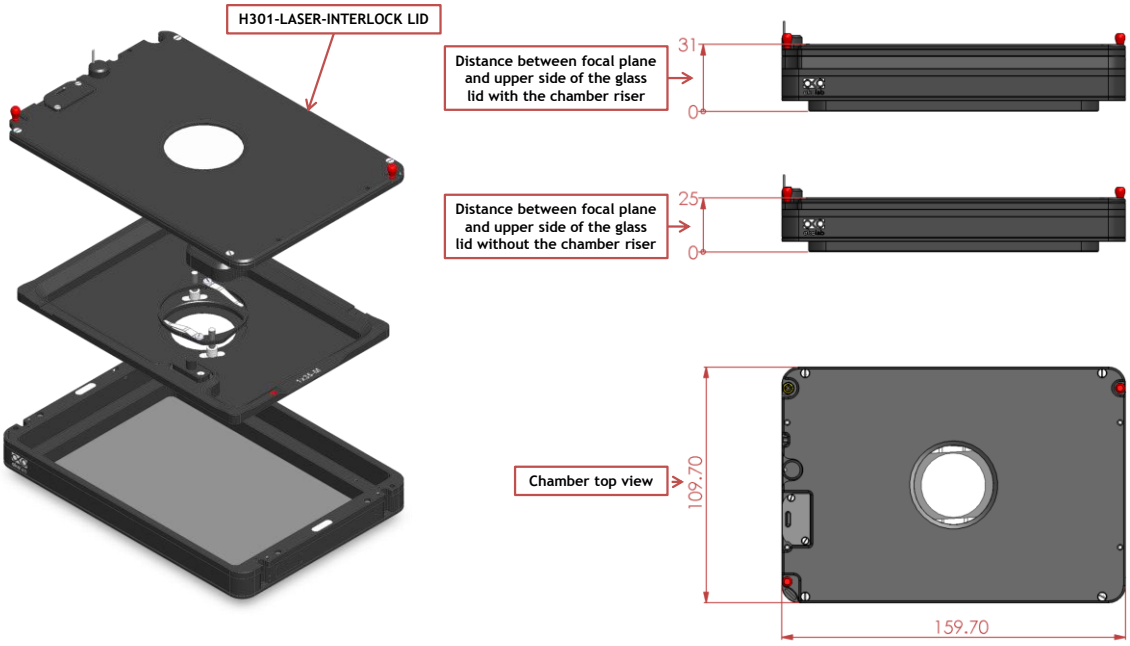
H301-LASER-INTERLOCK LID
Distance between focal plane and upper side of the glass lid with the chamber riser
31
0
Distance between focal plane and upper side of the glass lid without the chamber riser
25
0
Chamber top view
109.70
159.70

Figure 7. H301-NIKON-NZ100/200/500-N + H301-LASER-INTERLOCK-LID

4. Insertion of the Sample Feedback Temperature Sensor

Insert the Sample Feedback Temperature Sensor through the dedicated opening located in the H301-NIKON-NZ100/200/500-N (see Figure 8, Frontal and 3D views).

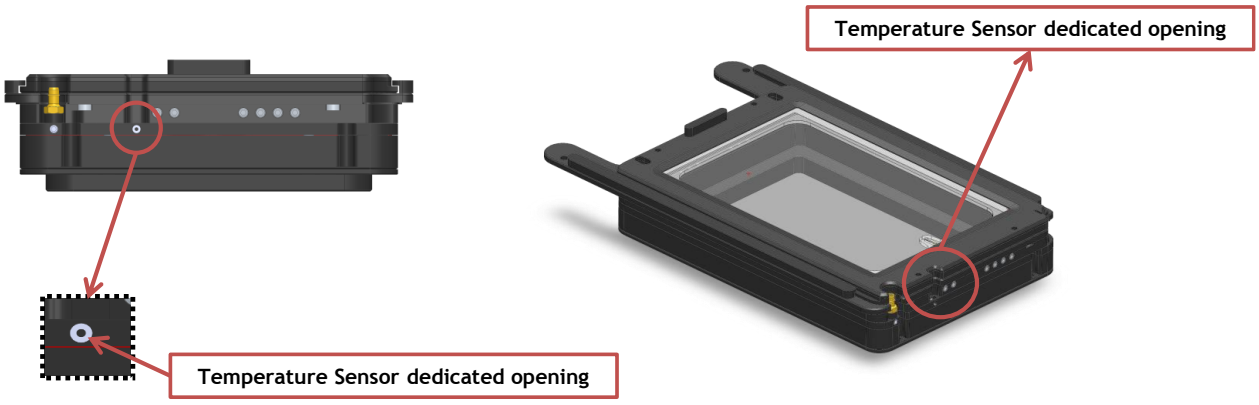

Figure 8. Insertion of the temperature sensor inside the chamber.

5. Insertion of Sample Holder into Chamber

Sample holders fit into the chamber base and are held in place by magnets embedded within both chamber and holder. To introduce a sample holder with the proper orientation, match the red dot on the holder to the one on the chamber base, as illustrated in Figure 9.

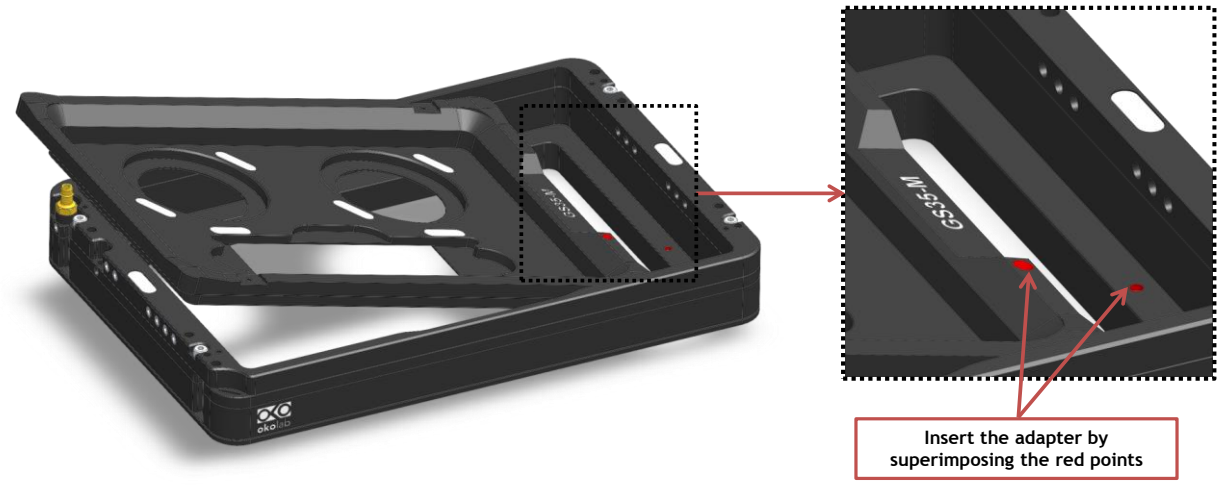

Figure 9. Introduction of the Specimen Holder inside the Chamber Base.

6. Working with 35 or 60 mm Petri Dish – Spacing Rings and Magnetic Locks

Magnetic locks prevent movement of 35 and 60 mm dishes inside the sample holder.

Figure 10 illustrates the available magnetic locks for 35 and 60 mm dishes. Threaded magnetic posts allow adjusting holder's height.

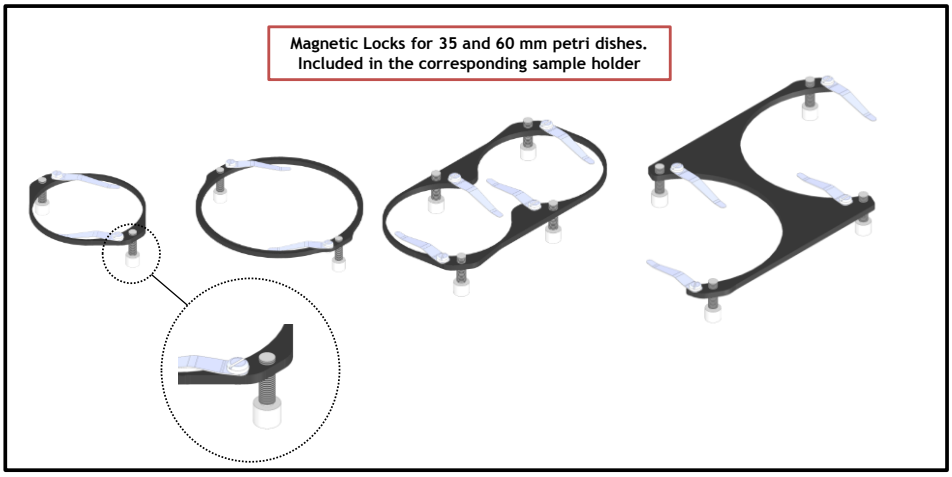

Figure 10.Magnetic locks for 35 and 60 mm dish

NOTE: Magnetic locks are included with sample holder.

Spacing rings accounting for variability in dish diameter from one manufacturer to another are also included. Figure 11 shows how to position the ring. Ring selection guide diagrams: rings for 35 and 60 mm dish -Figure 12 and Figure 13, respectively.

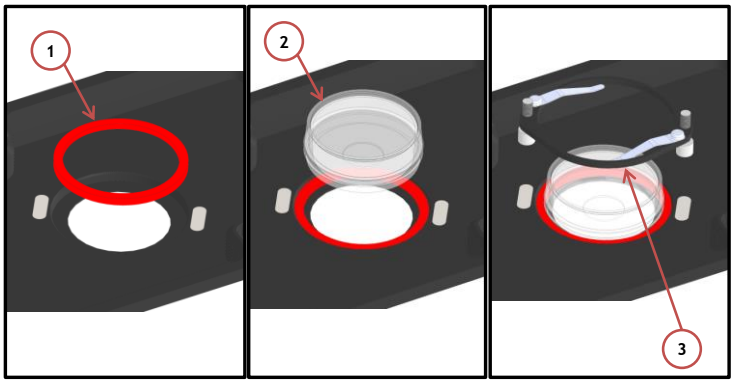

Figure 11. 1) Insertion of the ring, 2) insertion of the 35 mm dish, 3) insertion of the magnetic lock.

NOTE: Spacing rings are included with sample holder.

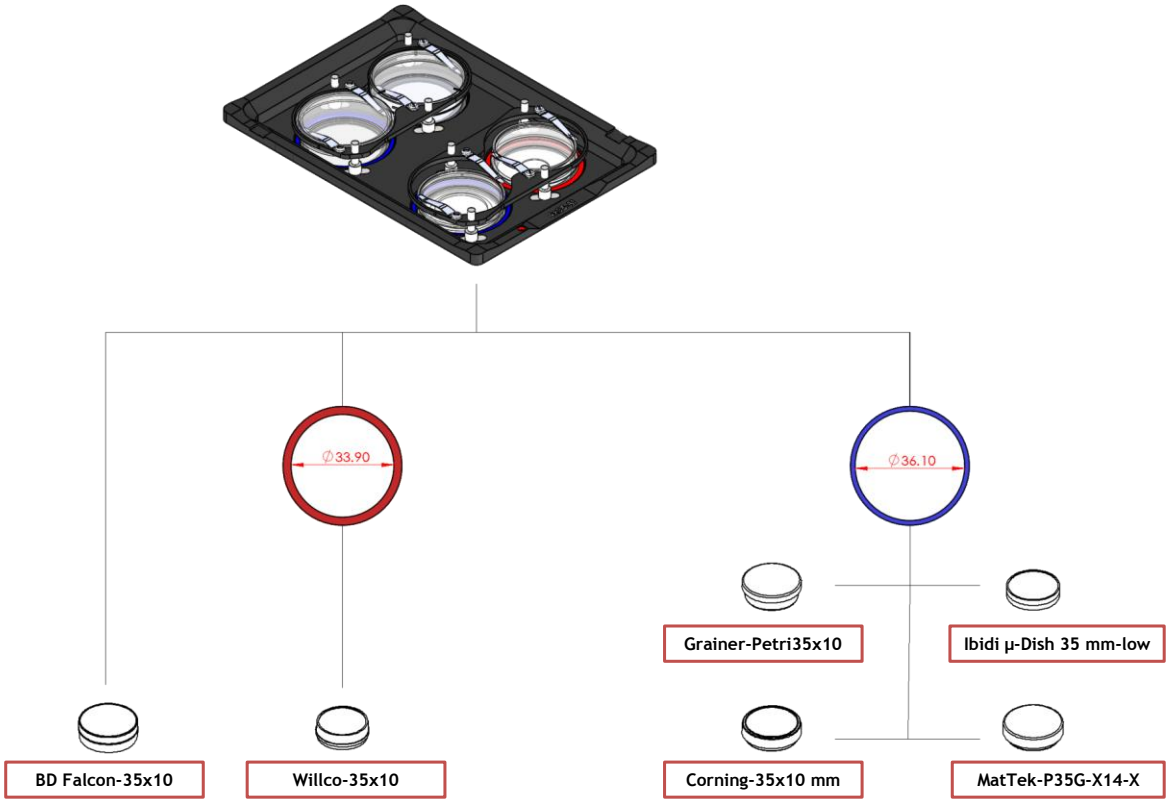

Figure 12. Ring selection scheme for 35 mm dish.

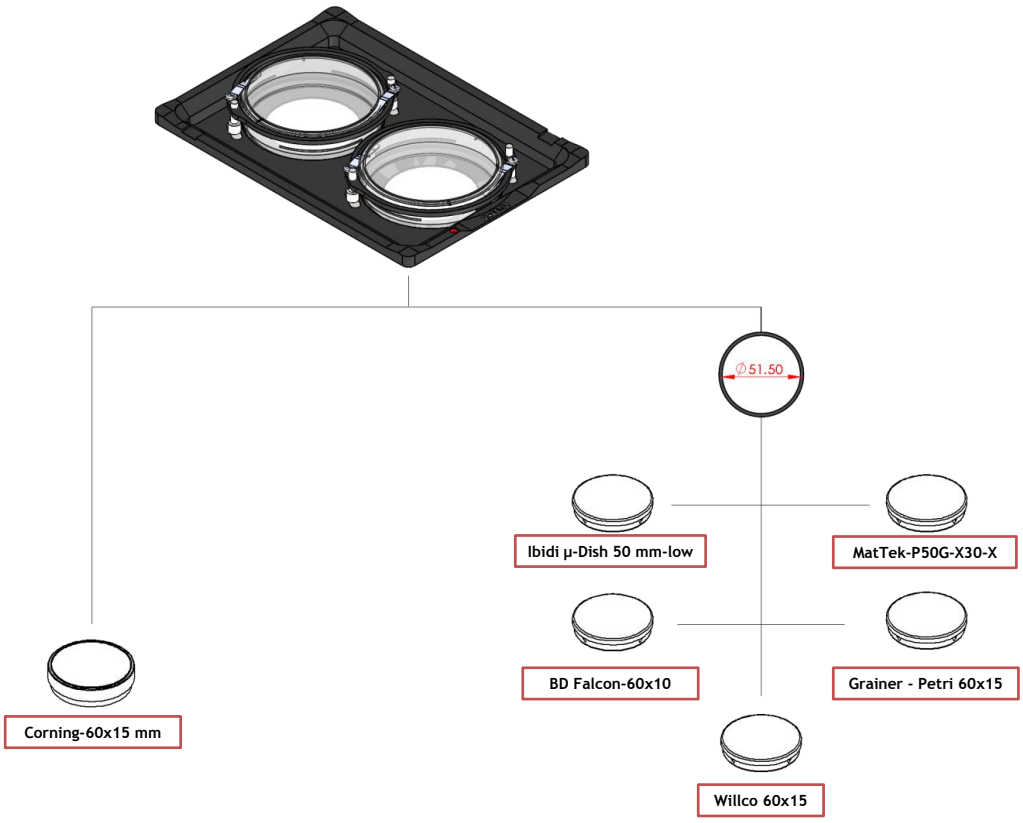

Figure 13. Ring selection scheme for 60 mm dish.

7. Working with 1x3'' and 1x2'' chamber slides - magnetic locks

Magnetic locks prevent movement of 1''x 3'' and 1''x 2'' chamber slides inside of the sample holder.

NOTE: Magnetic locks are included with sample holder.

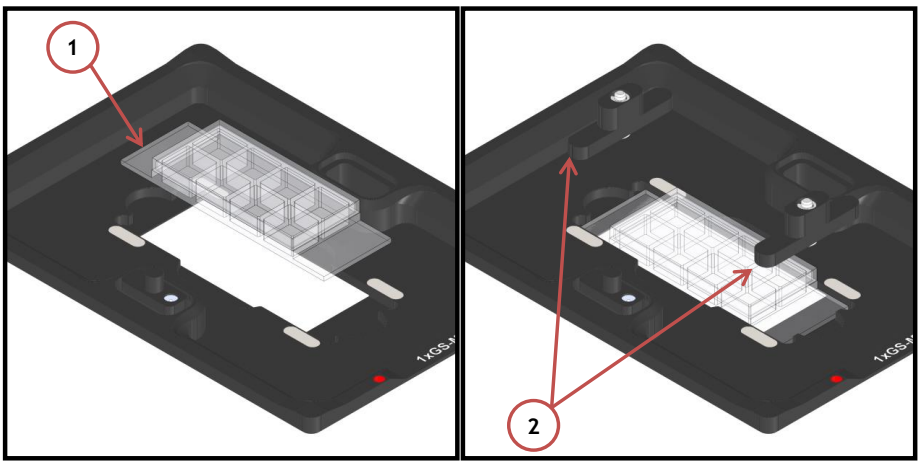

Figure 14. Magnetic lock for 1" x 3" chamber slide.

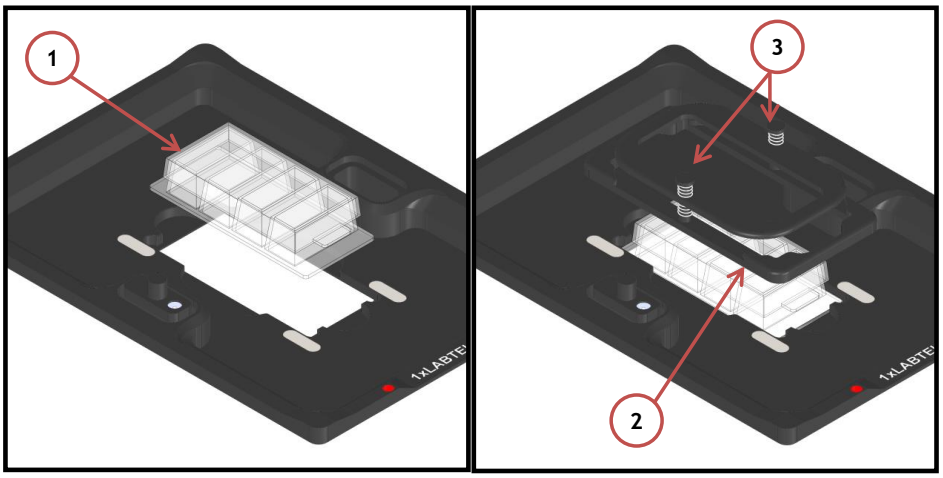

Figure 15. Magnetic lock for 1" x 2" chamber slide. To lock the slide, push simultaneously the buttons indicated with (3).

8. Working with MW Plates - Magnetic locks and Chamber riser

Magnetic locks hold MW plates in place into sample holder illustrated in Figure 16

NOTE: Magnetic locks must be ordered separately (NOT INCLUDED). Product codes: MW-LOCK 22.5 and MW-LOCK 16.5.

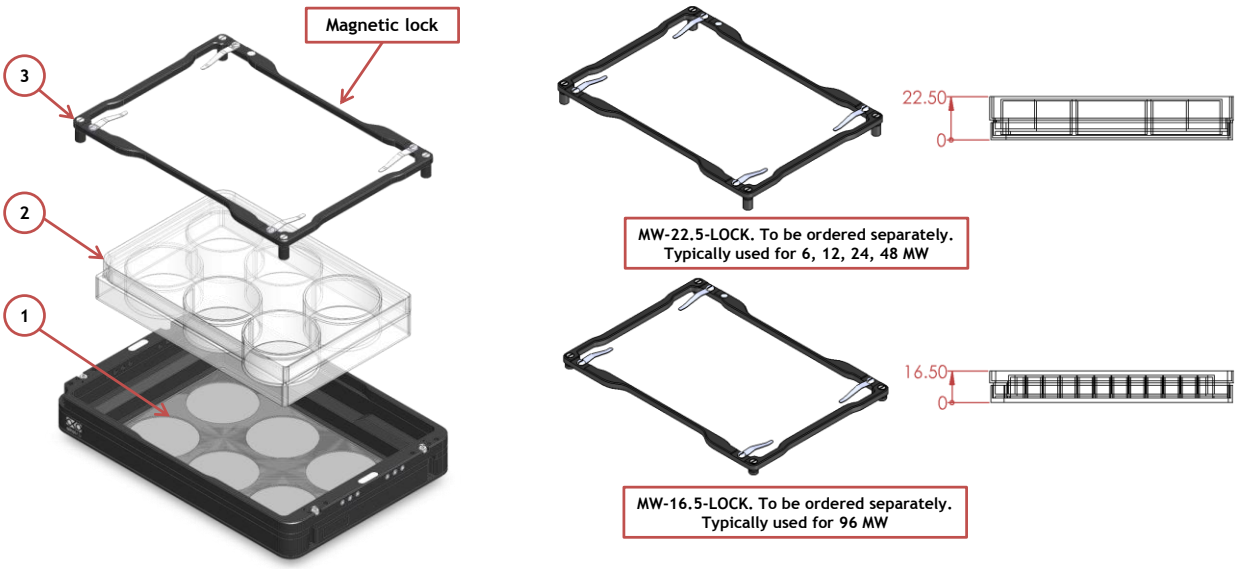

Figure 16. Magnetic locks for MW plates. To be ordered separately.

Chamber Riser increases chamber's height and is REQUIRED with 6, 12, 24, 48 MW plates, regardless of the Lid employed.

Figure 17 shows how to mount and screw the chamber riser. Screws location is indicated by letter A.

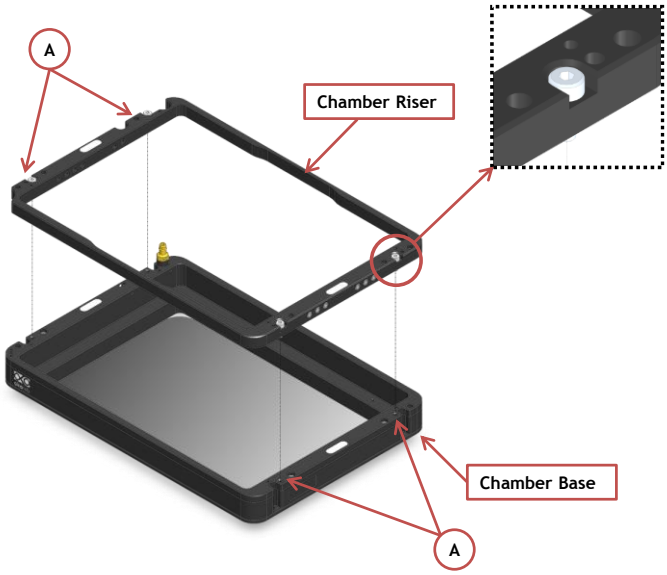

Figure 17. Riser Assembly.

9. Connection of the Gas Supply

A single silicon tubing carries output gas from the Okolab Gas Controller to the H301-NIKON-NZ100/200/500-N. Silicon tubing connects to a gas input - brass opening - located on a corner of the H301-NIKON-NZ100/200/500-N. See Figure 18. Connect by gently pushing silicon tubing onto brass opening.

Figure 18.Connection with gas supply.

10. Working with Perfusion

The Chamber Riser included with H301-NIKON-NZ100/200/500-N features 12 perfusion holes for the insertion of perfusion tubing up to 2.5 mm in outer diameter. Small screws plug the perfusion holes when not in use. (Grub screws M3x6). Remove small screws as necessary before introducing perfusion tubing.

NOTE: Working with Perfusion is possible with Mad City Labs NZ100/200 ONLY

Figure 19 shows location of perfusion holes.

NOTE: Magnetic locks are included with sample holder.

Figure 19. Perfusion

11. Connection of the Chamber with Z Piezo stage

Follow the steps shown in the images of Figure 20 and listed below in order to correctly connect the chamber with Z piezo stage.

1. Place the chamber on the stage and tighten 2 captive screws (See Image 2 of Figure 20). Captive screws housings are indicated with letter B in Image 2 of Figure 20.
2. Use a 1.5mm metric Allen Wrench to tighten one of the two captive screws (B in Image 3) while keeping the chamber sliding lid closed (See Figure 20 Image 3)
3. Tighten the other captive screw (B in Image 3) while keeping the chamber sliding lid open

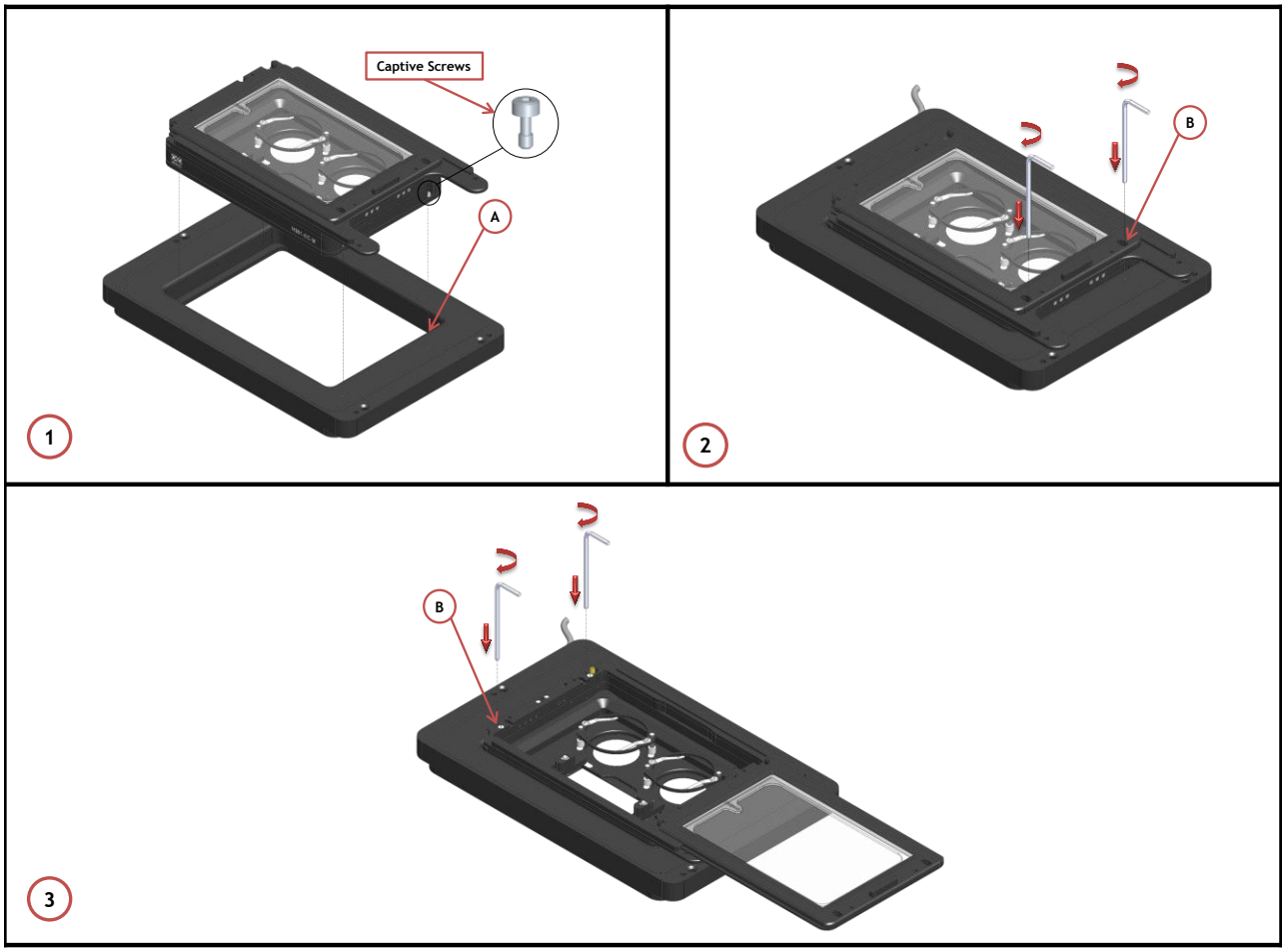

Figure 20. Connection of the Chamber with Z Piezo Stage